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TCR-Vβ Repertoire Analysis With RT-PCR Was Useful for the Early Detection of Pulmonary Relapsed T-Cell Lymphoma After Autologous Peripheral Blood Stem Cell Transplantation

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Pulmonary recurrence of malignant lymphoma is a rare event after stem cell transplantation. We report here a 45-year-old male who was successfully diagnosed with relapsed pulmonary T-cell lymphoma using an RT-PCR method. Clonal expansion of T cells expressing identical TCR V-D-J junction size (Vβ5-Jβ1.5) was demonstrated in lymphocyte groups obtained from both bronchoalveolar lavage fluid at relapse, and paraffin embedded lymph node samples resected when he was first diagnosed with angioimmunoblastic T-cell lymphoma. This method provided evidence to diagnose relapsed pulmonary angioimmunoblastic T-cell lymphoma in its early phase. Am. J. Hematol. 64: 124–127, 2000. © 2000 Wiley-Liss, Inc.

Key words: malignant lymphoma; pulmonary relapse; RT-PCR; TCR-β; BAL fluid

INTRODUCTION

Molecular analysis using a PCR technique is useful for detection of residual lymphoma lesions, or early relapsing lymphoma, after stem cell transplantation. Known genetic markers including chromosomal translocation, e.g., myc/IgH or bcl-2/IgH, are required to benefit from this highly sensitive method in a clinical setting [1–3].

T-cell lymphoma is the monoclonal proliferation of a certain TCR-bearing lymphocyte [5]. The determination of the V-D-J junction complementarity determining region-3 (CDR3) size patterns of TCR Vβ subfamilies using RT-PCR has been reported to be useful in demonstrating the clonally proliferated T-cell population [8]. In this report, we applied this method to obtain confirmation of relapsed angioblastic T-cell pulmonary lymphoma.

MATERIALS AND METHODS

Patient

A 45-year-old Japanese male was first noted to have generalized lymphadenopathies, and was diagnosed with Non-Hodgkin lymphoma, angioimmunoblastic T-cell lymphoma [5] (International Index; high-intermediate) from an inguinal lymph node biopsy specimen. He was admitted to our hospital in August 1996, and administered 4 courses of CHOP (cyclophosphamide 750 mg/m^2 on day 1, Adriamycin 50 mg/m^2 on day 1, vincristine 1.4 mg/m^2 on day 1, and prednisolone 100 mg PO on days 1–3) followed by 2 μg/kg of rhG-CSF (Filgrastin, Chugai, Japan) every 14 days, but he did not achieve clinical remission. After ESHAP (etoposide 40 mg/m^2 on days

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1–4, Cisplatin 25 mg/m^2 on days 1–4, cytosine arabinoside 2 g/m^2 on day 5, and methyl prednisolone 500 mg on days 1–5) salvage therapy followed by 2 μg/kg of subcutaneous rhG-CSF, his peripheral mononuclear cells were harvested. CD34$^+$ cells were isolated from the harvested mononuclear cells with an anti-CD34 monoclonal antibody coated flask and were cryopreserved. After myeloablative therapy with total body irradiation of 12 Gy 4 fractionation and administration 2.3 g/m^2 of etoposide, he received 8.5 × 10^6 CD34$^+$ cells/kg in December 1996. Engraftment was rapid, and he achieved complete remission. No residual disease was detected by computed tomography (CT) or gallium scan at the time of discharge in January 1997.

In March 1997, he was readmitted to our hospital because of pyrexia and exertional dyspnea. CT scan revealed diffuse ground-glass shadows in the bilateral lung fields, and a transbronchial biopsy specimen showed the infiltration of atypical CD3$^+$ lymphocytes in the lung parenchyma, although recurrent lymphoma was not pathologically confirmed. Further clinical and laboratory examinations suggested no signs of recurrent lymphoma.

Analysis of TCR Vβ Gene-Segment Repertoire and CDR3 Fragment Size

Cβ-, Vβ-, and Jβ-specific oligonucleotide primers corresponding to V(D)JC gene segments of the TCR β-chain were synthesized and used in the following studies as described by Genevée et al. [4].

Total RNA was extracted from lung mononuclear cells obtained from 10 mL bronchoalveolar lavage (BAL) fluid. Briefly, BAL cells were pelleted at 400*g* for 5 min at 4°C, followed by guanidinium thiocyanate–phenol–chloroform extraction. RNA was reverse transcribed to TCR-specific cDNAs using AMV polymerase (Gibco BRL, MD) in a reaction mixture containing Cβ 3′-oligonucleotide primers specific for the constant region of the TCR-β. The TCR cDNAs were amplified by 40-cycle PCR with 24 Vβ 5′-primers (Vβ1-w24) and the Cβ

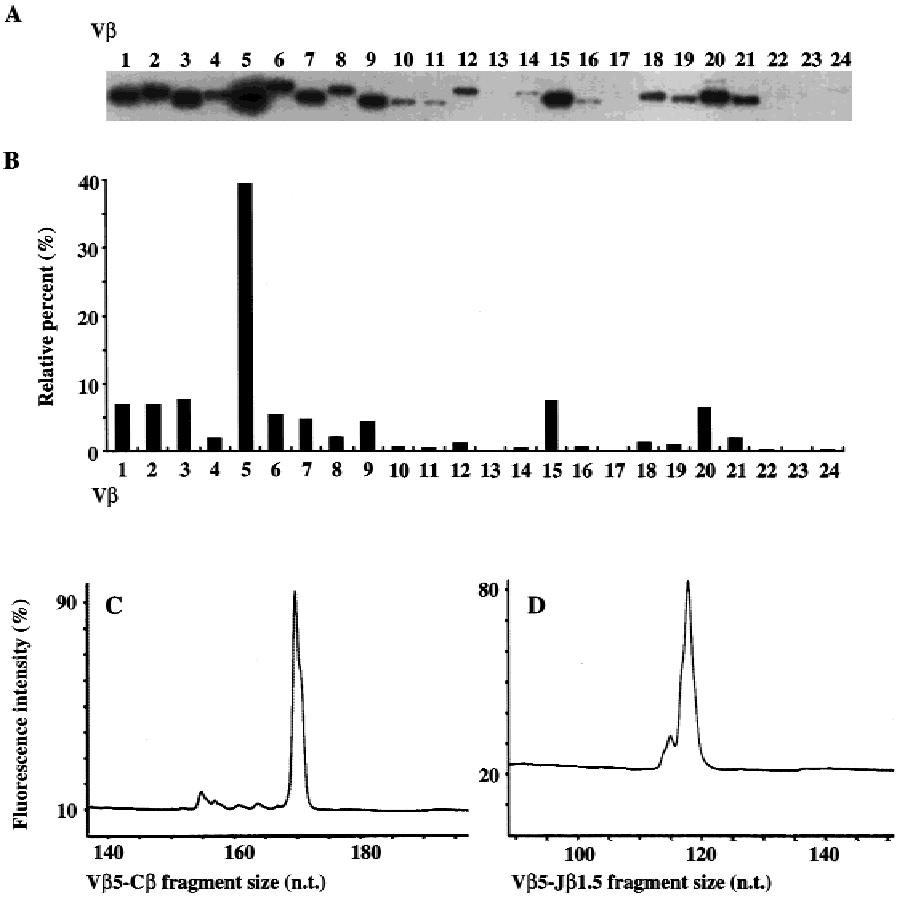

Fig. 1. Relative expression of TCR Vβ gene segments and clonal analysis of TCR Vβ5 in BAL fluid. RNA extracted from BAL fluid was reverse transcribed and amplified by 40-cycle PCR using 24 Vβ subfamily primers. The amplified products were detected by Southern blot analysis using a radiolabeled Cβ probe (A), and the value of each Vβ spot was expressed as a percentage of the sum of all Vβ signals (B). The clonal profiles (x axis, Vβ-Cβ size (C) or Vβ-Jβ1.5 size (D) in nucleotides; y axis, fluorescence intensity as described in Materials and Methods) of the Vβ5 subfamily strongly suggested clonal expansion.

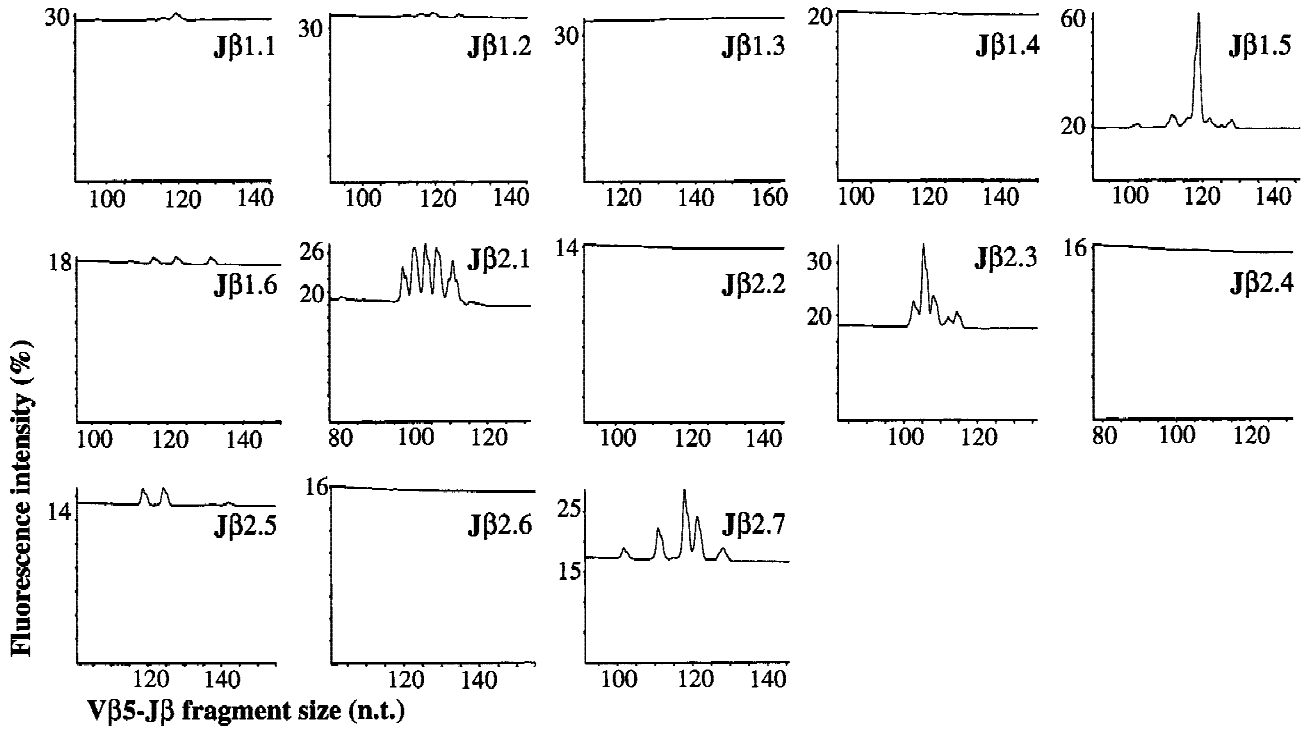

Fig. 2. Vβ5-Jβ size diversity in a paraffin-embedded lymph node. DNA extracted from a paraffin-embedded lymph node sample was amplified by 40-cycle PCR using paired Vß5 and one of each of the 13 kinds of fluorescent Jβ primers. The profiles obtained show Vβ5-Jβ size in nucleotides (x axis) and fluorescence intensity (y axis) of different amplified products.

3′-primer in the manufacturer's recommended PCR solution containing 1 U of "hot start" Taq polymerase (Ampli-Taq Gold; Perkin-Elmer Corp., Norwalk, CT). The amplified products were detected with Southern blot analysis using ^{32}P-labeled Cβ probe. Autoradiographs were visualized via computerized quantitative densitometry (BAS-2000II; Fuji Photo Film Corp., Tokyo, Japan).

To refine CDR3 size analysis, the Vβ5-Cβ PCR product from BAL cells was copied in a 5-cycle runoff reaction with a fluorescent labeled Cβ primer, or each of the 13 Jβ-fluorescent primers as described by Puisieux et al. [2]. Genomic DNA extracted from a few thin-sections of a formalin-fixed, paraffin-embedded lymph node by the Chelex method [6] was amplified by 40-cycle PCR with a fluorescent Jβ primer paired with the Vβ5 primer. The labeled PCR products were subjected to electrophoresis on an ALF DNA sequencer in the presence of fluorescent size markers and analyzed with a DNA fragment size program (Fragment Manager; Amersham Pharmacia Biotech Corp., Tokyo, Japan). The fluorescence intensities were expressed as 100% for the 3 fmol of fluorescent labeled DNA.

RESULTS AND DISCUSSION

To confirm the pulmonary relapse, we analyzed the transcripts encoding TCR β-chain repertoires and the CDR3 size length of Vβ-Cβ PCR products. TCR-specific cDNA, synthesized from BAL fluid by a reverse transcription reaction, was used in PCR amplification with 24 kinds of Vβ primers. Vβ-Cβ PCR amplification was followed by blotting and hybridization with a radiolabeled internal Cβ oligonucleotide primer. Figures 1A and 1B show the TCR Vβ gene expression of the T lymphocytes in the BAL compartment. TCR Vβ gene expression of the T lymphocytes was biasedly used as only a single TCR V-gene segment (Vβ5) with greater frequency (approximately 40% of the sum of the intensity of all the bands). To determine the CDR3 size distribution, Vβ5-Cβ PCR products were labeled with a fluorescent Cβ primer, or each of the 13 kinds of fluorescent Jβ primers. As shown in Fig. 1C, a single Vβ5-Cβ CDR3 size was observed, and only the Vβ5-Jβ1.5 fragment was amplified as the brightest monoclonal peak (118 nt) among 13 Jβ subsets (Fig. 1D).

The results of 40-cycle PCR with the Vβ5 primer and fluorescence-labeled Jβ primers on extracted genomic DNA from the paraffin-embedded lymph node sample showed the rearranged Vβ5-Jβ size diversities in a given Jβ subfamily (Fig. 2). Some results exhibited five peaks spaced by three nucleotides with a Gaussian distribution (such as Jβ2.1, Jβ2.3, and Jβ2.7), or faintly detectable multi-peaks. On the other hand, only the Jβ1.5 profile contained one dominant peak with an intensity 8 times

greater than the other peaks, highly suggestive of T-cell clonal expansion. Moreover, the size of the clonal Vβ5-Jβ1.5 amplified fragment (118 nt) exactly matched that of the dominant Vβ5-Jβ1.5 fragment from the BAL compartment. All of these results were compatible with recurrent malignant lymphoma in the lung. According to these results, we started 40 mg/day of prednisolone orally. His abnormal lung shadow disappeared, and he was discharged 20 days after the start of prednisolone. He was, however, readmitted for pneumocystis carinii pneumonia 10 days after discharge. His BAL fluid at that time exhibited a polyclonal TCR-Vβ repertoire suggestive of infection, not relapsed lymphoma (data not shown). His pneumonia was successfully treated with ST this time. These results, altogether, suggest the reliability of this diagnosis technique.

There have been two cases reported in which a similar rare pattern of relapse was exhibited [7,9]. The authors suspected that the relapse was caused by the reinfusion of minimal lymphoma cells contaminated in the transplanted non-purged hematopoietic stem cells because of multifocal pulmonary recurrence. Our patient, however, received only $CD34^+$ PBSC. Thus, it is unclear whether the lung relapse was caused by the infusion of minimal residual lymphoma cells contaminated in CD34 positive cells, or minimal residual lymphoma cells in the lung. Further clinical studies are required to elucidate the clinical benefits of this purging method in lymphoma patients.

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